APPLICATION NOTE

NIS.ai AI module for microscopes

Label-free detection of neuron and astrocyte regions using deep learning technology

When co-culturing multiple types of cells, it is generally necessary to label the cells with a marker molecule to identify the cell type (Reference 1). However, immunostaining requires the fixation of cells, which is incompatible with live observation. In addition, toxicities during transgenesis and fluorescence observation may become an issue when visualizing living cells with a fluorescent protein.

NIS.ai (Segment.ai), a deep learning module of NIS-Elements imaging software, is a technology for detecting specific cell regions that are difficult to extract with conventional binarization and image processing. This method makes it possible to detect cell types from unlabeled images.

In this application note, Segment.ai shows an example of quantitative analysis of the cell regions of neurons and astrocytes in differential interference contrast (DIC) images of co-cultured samples of these cells, conducted with the cooperation of Dr. Tomomi Nemoto, director of the Exploratory Research Center on Life and Living Systems, National Institutes of Natural Sciences, and Dr. Hirokazu Ishii and Dr. Motosuke Tsutsumi of the Center's Biophotonics Research Group.

Keyword: Segment.ai, label-free analysis, neurons, astrocytes

Methods

A co-cultured sample of neurons and astrocytes was fixed with 4% paraformaldehyde after 10 days cultivation and labeled with immunostaining using the synaptophysin antibody, which is a neuronal marker. DIC images of the sample were acquired and used as images for training and inference. From the acquired DIC images, a ground truth binary of the total cell region was created (annotation), and the neuron region was annotated with reference to the fluorescently-labeled synaptophysin image to prepare training data (40 sets). Using these data, we trained Segment.ai with 1000 iterations to create a trained Segment.ai model. This model was applied to target DIC images (Fig. 1).

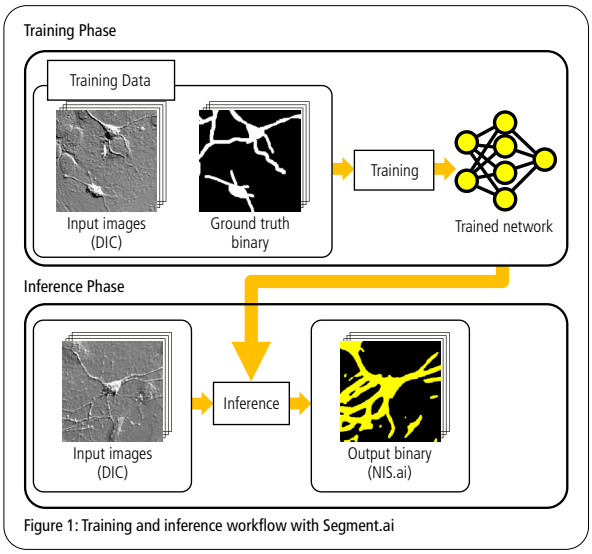
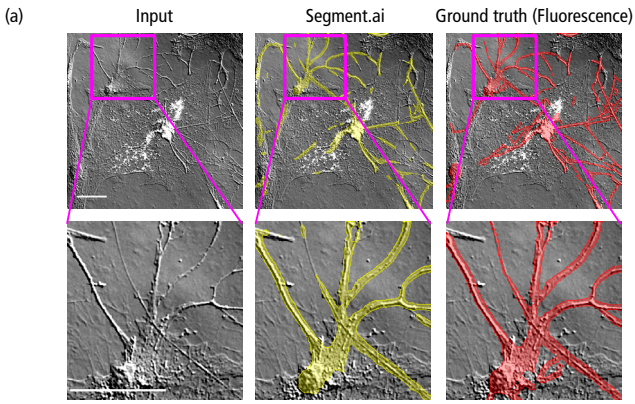
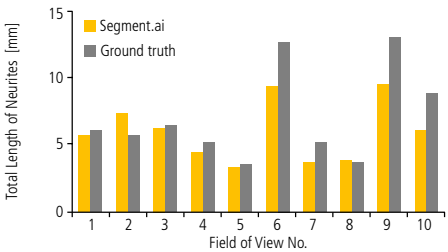


Figure 1: Training and inference workflow with Segment.ai





(c)

	Accuracy [%]
Average	82.4
SD	12.5

Figure 2: Detection of neuron regions from DIC images

(a) Comparison of the neuron region detected using Segment.ai (middle) and the manually annotated ground truth neuron region (right). Input: DIC images input to Segment.ai. Segment.ai: Binary (yellow) of the neuron region inferred and output from the input. Ground truth: Ground truth binary (yellow) of the manually annotated neuron region. The lower row are enlarged images of the upper row images. Scale bars: 50 µm

(b) Total length of neurites [mm] obtained for the 10 fields of view used for inference. Yellow: Quantitative results using the output of Segment.ai. Gray: Quantitative results using the ground truth binary.

(c) Detection accuracy for the 10 fields of view used for inference [%]. The accuracy was calculated by the following formula:
Accuracy [%] = 100-(| 100- [Segment.ai quantitative results] * 100 / [Ground truth quantitative results] |)

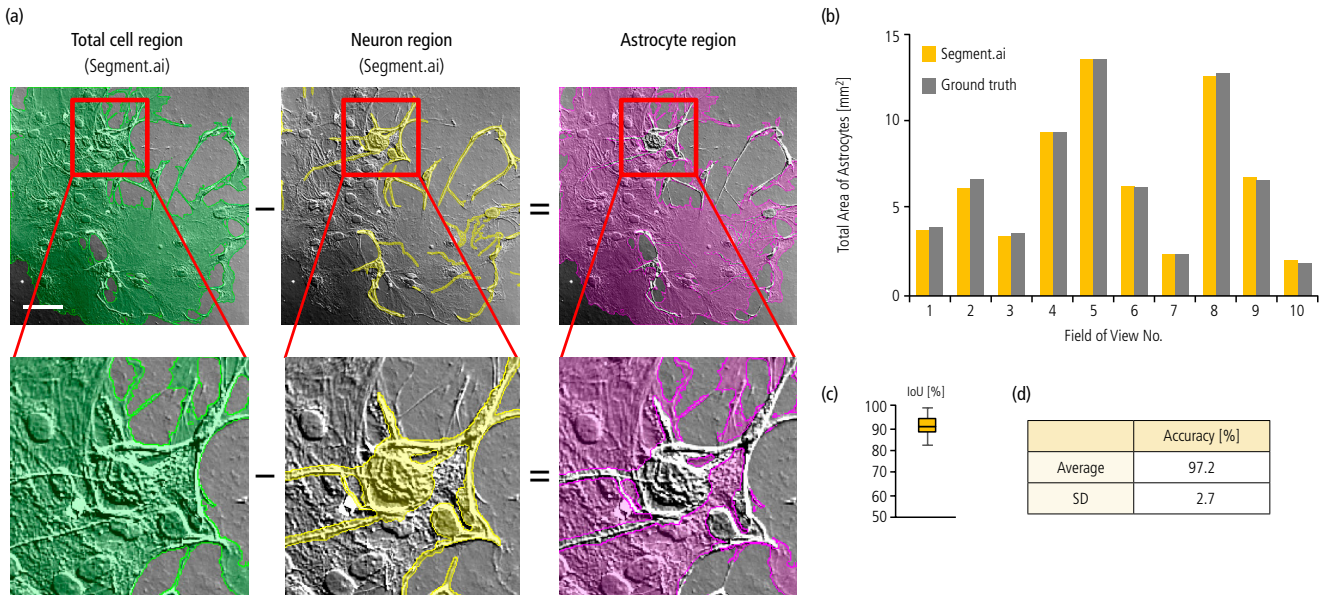


Figure 3: Detection of astrocyte region from DIC image

(a) A method for detecting the astrocyte region. The astrocyte region (right) was identified by subtracting the neuron region (middle) from the total cell region (left), both detected by Segment.ai. The lower row are enlarged images of the upper row images. Scale Bar: 50 µm

(b) Total astrocyte area [mm^2] obtained for the 10 fields of view used for inference. Yellow: Quantitative results using the output of Segment.ai. Gray: Quantitative results using the ground truth binary.

(c) Box plot of intersection over union (IoU) for the 10 fields of view used for inference. IoU is an index for evaluating the overlap between the ground truth regions and the inferred regions, and was calculated by the following formula (an accurate result will be close to 100%):

IoU [%] = 100 * [Intersection area of Segment.ai detection region and Ground truth region] / [Union area of Segment.ai detection region and Ground truth region]

(d) Detection accuracy for the 10 fields of view used for inference [%]. The accuracy was calculated by the following formula:

Accuracy [%] = 100-(| 100- [Segment.ai quantitative results] * 100 / [Ground truth quantitative results] |)

Results

By applying the above-mentioned trained Segment.ai model, we attempted to detect the regions of neurons and astrocytes using only the DIC images of co-cultured samples of these cell types (Fig. 2a).
A comparison of the neurite lengths between the detected neuron region and the ground truth neuron region showed that they matched with an accuracy of 82.4% (Fig. 2b, 2c). Furthermore, we also attempted to detect the astrocyte region by subtracting the neuron region from the total cell region using the Segment.ai model that detects the total cell region from the DIC images (Fig. 3a). As a result, it was confirmed that the astrocyte region was also quantified with high accuracy (97.2%) in the area comparison with the ground truth (Fig. 3b, 3c, 3d).

Discussion and Summary

By utilizing NIS.ai, each region of neurons and astrocytes could be identified by inputting DIC images alone, and the neurite length and cell area could be quantified with high accuracy.

Studies about neurons may require long-term cultures of more than 10 days (Reference 2). Since this method enables label-free analysis in time-lapse observation of such samples, it is expected to be applied to minimally invasive culture experiments that avoid the cytotoxicity caused by labeling.

Materials

Cells

- Mouse-derived primary neurons and astrocytes (co-culture)

Antibody

- Anti-synaptophysin antibody (neuron marker) (Rabbit, GeneTex, # GTX100865, 1: 400)
- Alexa Fluor 488-labeled anti-rabbit IgG antibody (Goat, Invitrogen, # A11034, 1: 200)

Imaging/analysis conditions

- Microscope: Eclipse Ti2-E
- Objective: CFI Plan Apochromat VC 20X
- DIC system:
  Module: LWD DIC N1 Dry TC-C-ML-N1D
  Slider: DIC PF ELWD 20xC T-C
- CMOS camera: DS-Qi2
- Software: NIS-Elements AR v5.40.00
- GPU: NVIDIA GeForce RTX 2080 Ti

References

1. N. Goshi *et al*., "A primary neural cell culture model to study neuron, astrocyte, and microglia interactions in neuroinflammation". *J. Neuroinflammation*. 17:155. (2020).
2. R. T. Roppongi *et al*., "Low-density primary hippocampal neuron culture". *JoVe*. doi:10.3791/55000. (2017)

Product informatŠn

NIS.ai module for microscopes

The NIS.ai image processing/analysis module, which extends the functionality of NIS-Elements imaging software, provides four types of AI modules: Enhance.ai, Convert.ai, Segment.ai, and Clarify.ai.

The Segment.ai module used in this application note can be trained to segment only regions such as the cells and structures that you want to analyze. This provides new solutions for cases where target extraction is difficult with conventional binarization and where manual classification was previously required.